

The Test Tube and Slide Methods In the Rh₀ Testing Procedure

—Georgia's Comparative Study—

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ABOUT 5 years ago, in the Georgia Department of Public Health, the division of laboratories acceded to the request of the division of maternal and child health to establish the Rh test as a routine function. Use of the tests, however, would be limited to prenatal and immediately related cases.

During the period of planning the work, it was questioned whether the Rh test should be included in a public health program, but it was decided that use of this test could be one of the connecting links between the public health and clinical laboratory fields. Use of the test for transfusion purposes undoubtedly belongs to the hospitals or clinics, while its utilization for giving information relating to erythroblastosis or hemolytic disease in the newborn is of public health importance. The participation of the Georgia State laboratories in this procedure, it was decided, would be confined to testing for the Rh₀ factor, the most potent and important of the Rh (Rhesus) antigens. Rh

typing and the performance of sensitization tests or titrations could be obtained as required in hospital and private laboratories. It was thought that the adoption of this program would do much toward bringing the importance of this recently discovered blood constituent to the attention of the medical profession.

The question then arose as to the technique to be employed. Since the discovery of the Rh factor by Landsteiner and Wiener (1) in 1940, much has been written about its importance and the laboratory methods for its detection. In searching the literature for methodology, we found some confusion as to the most reliable test. The most desirable procedure would be one which could be applied to clotted specimens of blood submitted for the serologic test for syphilis as required by law in prenatal cases, and which could easily be adapted to a large volume of work.

The Two Techniques

The test tube method devised and suggested by Dr. Louis K. Diamond of Children's Hospital, Boston, was selected. An experienced technician was sent to Dr. Diamond's laboratory for training in this procedure. During the period of preparation for this work, we were spared the experience of some laboratories

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in regard to the low specificity of anti-Rh serum prepared from rabbits, since more specific anti-serum from human sources had become available.

The selected technique consisted of the utilization of 1 drop of a 2-4-percent cell suspension in its own serum with 1 drop of anti-Rh₀ serum in a test tube and incubation in a 37° C. water bath for 30 minutes. The tests were then centrifuged for 1 minute at 1,000 r.p.m. The results were read macroscopically under suitable illumination and against a white background. Tests showing indefinite or negative agglutination were checked by low-power microscopic examination of a drop of the mixture placed on a glass slide. A drop of saline was added to the mixture on the slide if rouleaux formation was suspected.

Soon after this work was begun, we would receive an occasional report of a disagreement between our laboratory and some hospital or private laboratory which had begun the Rh test work in conjunction with transfusions. This caused concern. Having but short experience, we thought it well to begin investigating the conditions which might influence the results of this test.

Although we had been assured that this technique could be applied to clotted specimens of blood received through the mail, we began to wonder about the effect of hemolysis on the Rh test. Also, we had noted some difference in the potency of different lots of anti-Rh₀ serum. This product could be obtained only in small quantities at that time, and, therefore, change in lots frequently occurred. We were also concerned as to what constituted a drop, or if differences in the size of drops of antiserum would have any effect on the results.

The age of the specimen might be responsible for some of the discrepancies, it was also thought. Hospital or private laboratories usually performed the test on freshly collected specimens, whereas the specimens reaching the health department laboratories were probably from 1 to 3 days old. Investigations were begun in order to obtain information on these questions.

As a result, it was found that damage to some of the cells indicated by hemolysis, reflected in 120 specimens a more significant influence

on the results than did the age. The strength of the cell suspensions ranging from 1 to 4 percent did not appear to effect appreciably the results in 166 specimens. Parallel tests on 436 specimens showed a difference in potency of different lots of anti-Rh₀ serums.

In routine work, Rh₀ test results are reported as positive or negative. For finer comparisons in these investigations, however, the results were recorded in "plus" terminology. Four plus (4+) represents complete agglutination, and minus (-) represents absence of agglutination. Partial agglutination in varying degree is designated by numerical values between these extremes.

The results of the study with two different lots of anti-Rh₀ serums is presented in table 1. The high percentage of negatives in this series is due to the selection of the specimens.

Table 1. Comparison of reactivity of 2 lots of anti-Rh₀ serums by the tube method on 436 specimens

Number of specimens	Anti-Rh ₀ serum	
	Lot 1	Lot 2
292-----	4+	4+
32-----	3+	4+
9-----	2+	4+
5-----	2+	3+
1-----	1+	4+
97-----	—	—

A slide test procedure was being used by most laboratories in the southeastern region of the United States in their Rh testing, we learned. So, in parallel with our test tube procedure, we began to use the slide test developed and recommended by Dr. John Elliott of the blood bank of Dade County, Fla. Dr. Elliott visited the Georgia laboratories, demonstrated his technique, and instructed in its performance.

The slide test technique, also used extensively in the hospitals in Georgia, consisted of the mixing of 1 drop of anti-Rh₀ serum and 2 drops of a 40-50-percent cell suspension in its own serum on a glass slide. The slide is then placed on a view-box equipped with a special light bulb which supplies heat essential for the reaction and at the same time provides good illumi-

nation for reading the results. The view-box is tilted back and forth slowly, and the results are read at the end of 2 minutes.

The Comparative Studies

Six hundred and four specimens were examined in parallel by the test tube and slide methods as shown below:

	<i>Number of specimens</i>
Total agreement-----	600
Complete agreement—positive-----	496
Complete agreement—negative-----	104
Total disagreement-----	4
Positive by tube—negative by slide-----	0
Negative by tube—positive by slide-----	4

Disregarding 1+ reactions, as suggested by both Dr. Diamond and Dr. Elliott, 600 (99.3 percent) of the specimens showed complete agreement. Four (0.7 percent) specimens were negative by the test tube method and positive by the slide method. These 4 specimens also gave positive results by the test tube technique when another anti-Rh₀ serum was employed.

Another comparative study of the tube and slide methods employing antisera from 2 different sources was made on 2,010 specimens. These anti-Rh₀ sera were recommended for use in either the tube or slide procedures. The disagreements are given in table 2, and the agreements are shown in the following tabulation:

	<i>Number of specimens</i>
Complete agreement-----	1,978
Positive (4+) reactions-----	1,711
Negative (—) reactions-----	267
Relative agreement-----	22
Positive (2+, 3+, 4+) reactions-----	13
Negative (± and —) reactions-----	9

Complete agreement was obtained in 1,978 (98.4 percent) of the specimens in this series; relative agreement in 22 (1.1 percent). Ten (0.5 percent) specimens showed some difference in degree of agglutination either in methods of testing or in lots of anti-Rh₀ serum used. This study reveals a closer agreement in Rh₀ testing than is usually achieved by the application of different techniques in many of the various types of laboratory examinations.

Table 2. Disagreement in Rh₀ results on 2,010 specimens tested by tube and slide methods with anti-Rh₀ sera from 2 sources

Tube method		Slide method		Disagreement	
Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
4+	2+	4+	1+	1	-----
2+	±	4+	4+	1	-----
2+	±	—	±	-----	1
2+	—	—	—	-----	1
1+	—	—	—	6	-----
Total (10)-----				8	2
				Total number of disagreements----- 10	
				Total percentage of disagreements----- 0.5	

Using Both Procedures

Following these investigations, it was felt that for better results in Rh₀ testing, both the tube and slide methods should be employed. The slide test technique was, therefore, adopted as a supplementary procedure on all specimens giving a negative or slightly positive reaction by the tube method. A negative report for a specimen indicates failure to show any agglutination by either of these methods. As a result, reports of discrepancies between the Georgia health department laboratories and other laboratories have become rare.

We occasionally find a specimen which gives a slight agglutination by one method and no agglutination by the other method. Sometimes a specimen is negative by the tube method and shows a slight agglutination at the end of 2 minutes by the slide test. We were instructed to make readings at the end of 2 minutes and to disregard the clumping which occurs after that time. This, however, is difficult to do, when increased agglutination is observed at the end of 3 minutes and becomes maximum at the end of 4 minutes. Such specimens are rechecked by the tube method, using a different lot of anti-Rh₀ serum. An occasional specimen is observed to give a slight agglutination by the tube method but is negative by the slide test.

In such disagreements, both tests are repeated to rule out possible error. If the discrepancy still exists, a letter accompanies the report, explaining the omission of the Rh₀ test results and suggesting the submission of another speci-

men for repetition of both tests. If the subsequent specimen shows a disagreement between the 2 test methods, the report is usually marked positive with the further suggestion that the more critical Rh typing be made on that person preparatory to transfusion.

Sources of Error

Errors in test performance are always contributing factors to disagreements in laboratory examinations. Sources of error common to both the tube and slide methods of Rh testing are:

Use of anti-Rh serum after its expiration date, or when it has become contaminated.

Performance of test without due consideration of temperature.

Mistaking for agglutination red blood cells enmeshed in particles of fibrin.

Performance of tests on specimens of blood which are badly hemolyzed or grossly contaminated.

In the tube method, a too vigorous shaking breaks up the agglutination and leads to errors in reading results. Drying processes and rouleaux formation in the cell suspension and anti-Rh serum mixture are sometimes mistaken for agglutination in the slide method.

Laboratory technicians quite often become confused by the arguments of the advocates for the tube and slide test techniques in Rh testing in regard to the superiority of one of these methods. The choice of method, however, is probably less important than the ability and skill of the technician who performs the test.

Every technician should receive adequate training in the Rh test before being given the responsibility of performing it and reporting the results independently. It must always be kept in mind that disagreement in results may occur at any time in the same laboratory or in different laboratories if rigid adherence is not given to prescribed techniques.

Summary and Conclusion

In the division of laboratory services of the Georgia Department of Public Health, studies were made to determine the causes of discrepancies in Rh₀ test reports from different laboratories. The tube and slide test methods were performed in parallel on large series of specimens of blood. Results with lots of anti-Rh₀ serums from two different sources are compared. Although these studies reveal a closer agreement than is usually achieved in many types of laboratory examinations, the slide test method has been adopted as a supplementary procedure to the tube method for confirmation in negative or doubtful reactions.

In Rh testing, deviations from prescribed techniques probably contribute more to disagreements in results among laboratories than does the difference in the reactivity levels of the tube and slide methods.

REFERENCE

- (1) Wiener, A. S.: Blood groups and transfusion. Ed. 3. Springfield, Ill., Charles C. Thomas, 1946.

PHS Staff Announcement

Leonard M. Board, chief of the Sanitation Program, Division of Sanitary Engineering Services, has been assigned to duty in Lima, Peru, as regional sanitary engineer advisor for the Institute of Inter-American Affairs of the Foreign Operations Administration. In his new position Mr. Board will give advisory service in sanitary engineering matters to the health missions in 18 South and Central American countries. Mr. Board was commissioned in the Public Health Service in 1943.